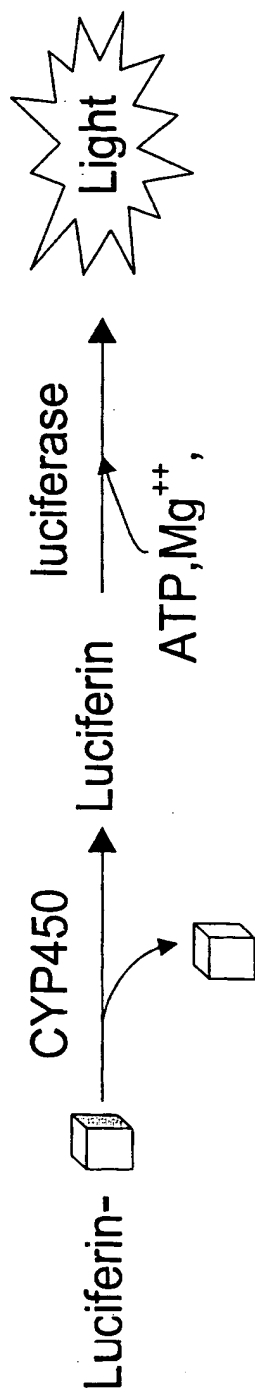


Figure 1



### Examples

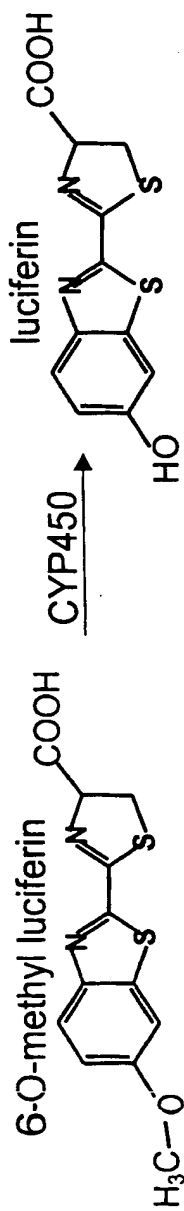
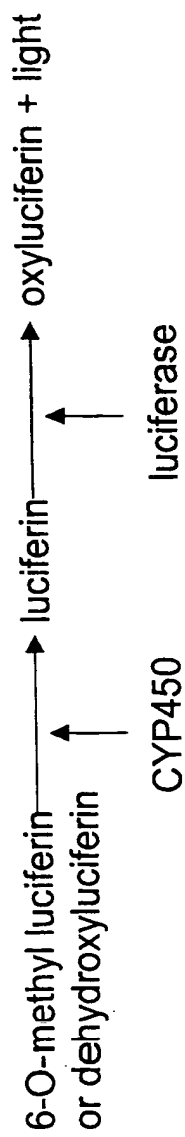
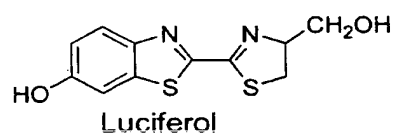
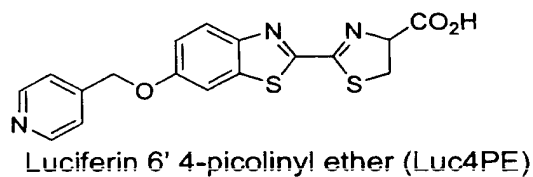
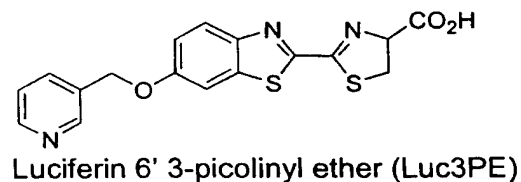
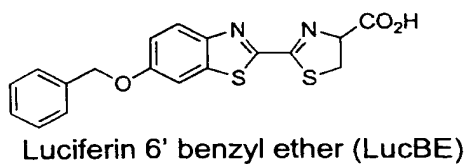
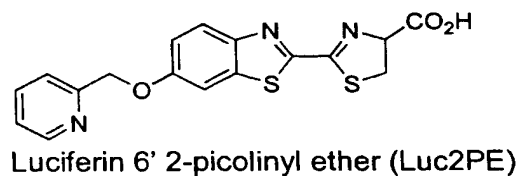
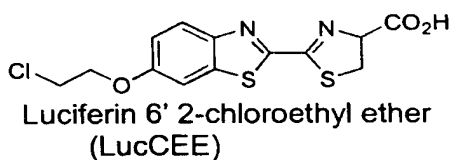
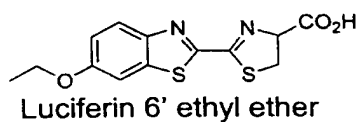
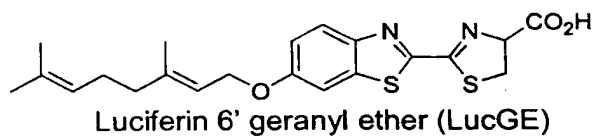
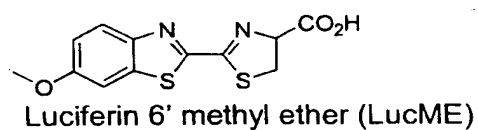
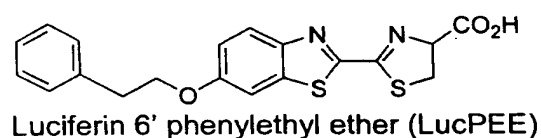
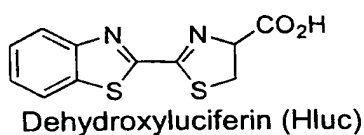
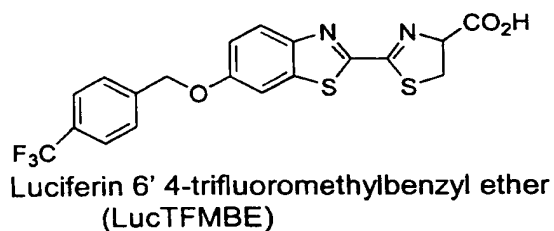
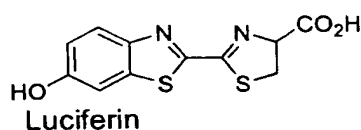


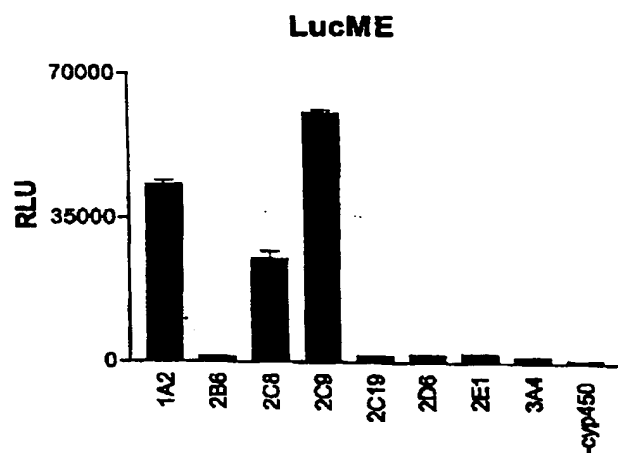
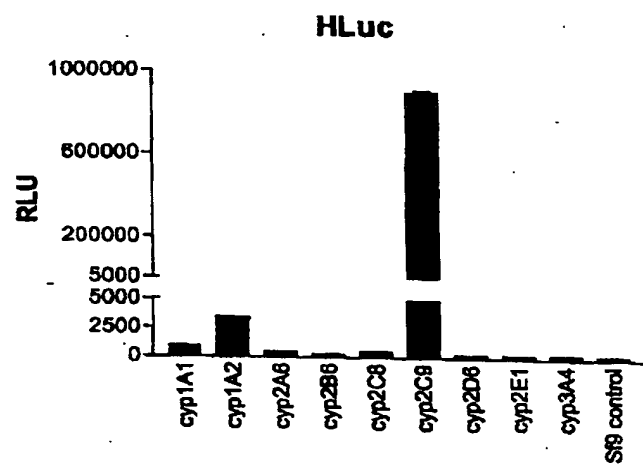
Figure 2

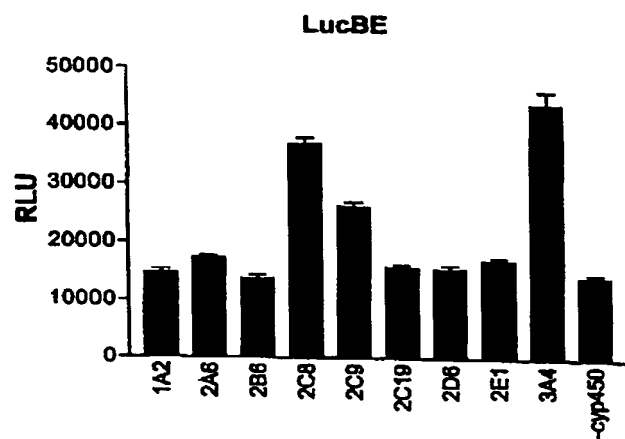
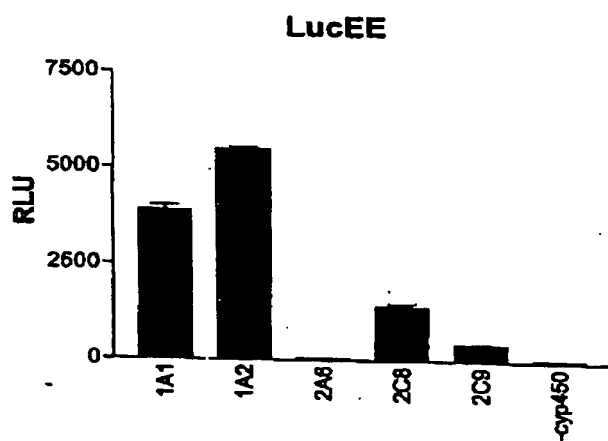
D-luciferin and D-luciferin derivatives.



**Figure 3**

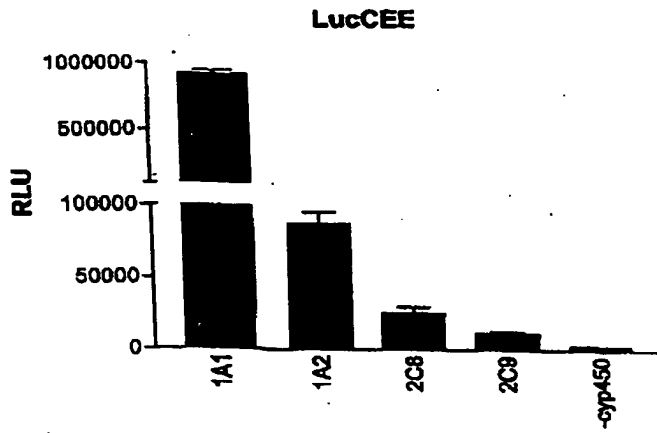
Two-step luminescent cyp450 reactions using D-luciferin derivatives

**A****B**

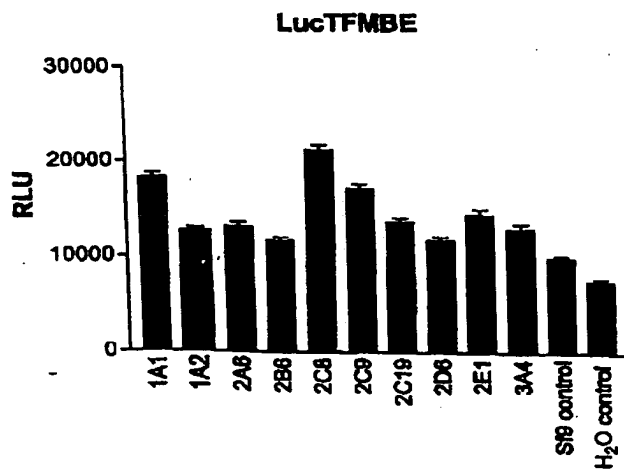
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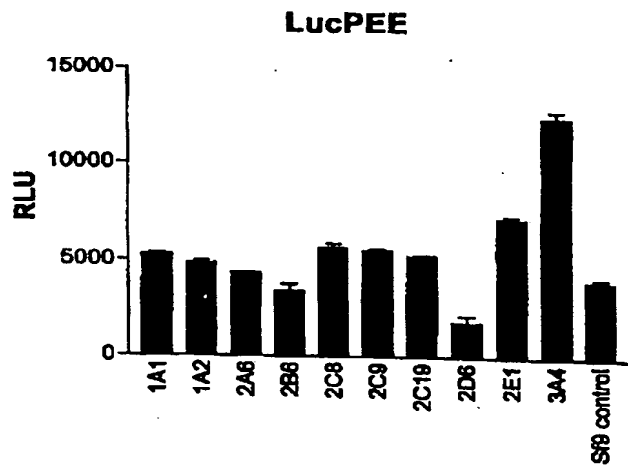
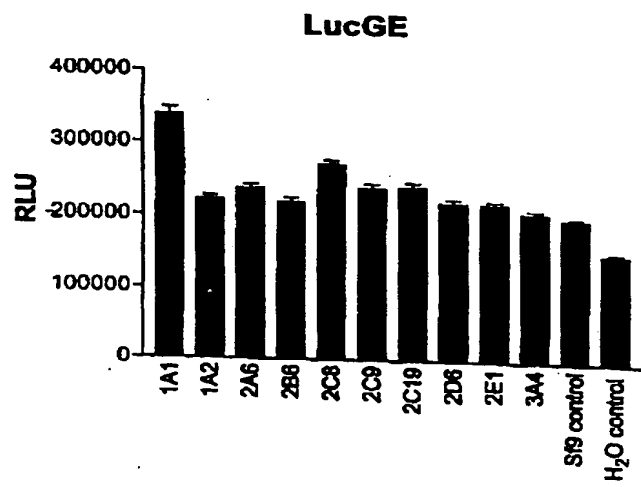
5/26.

**E**

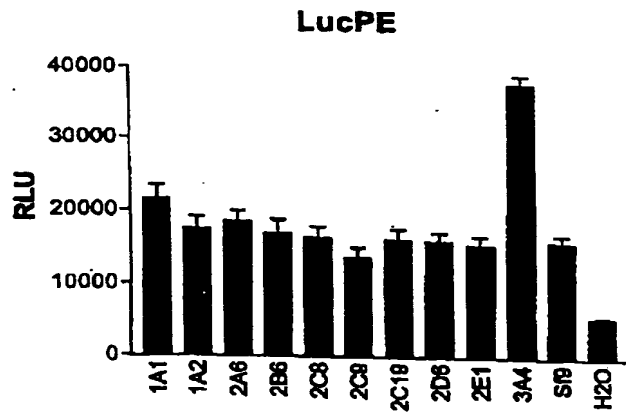


**F**



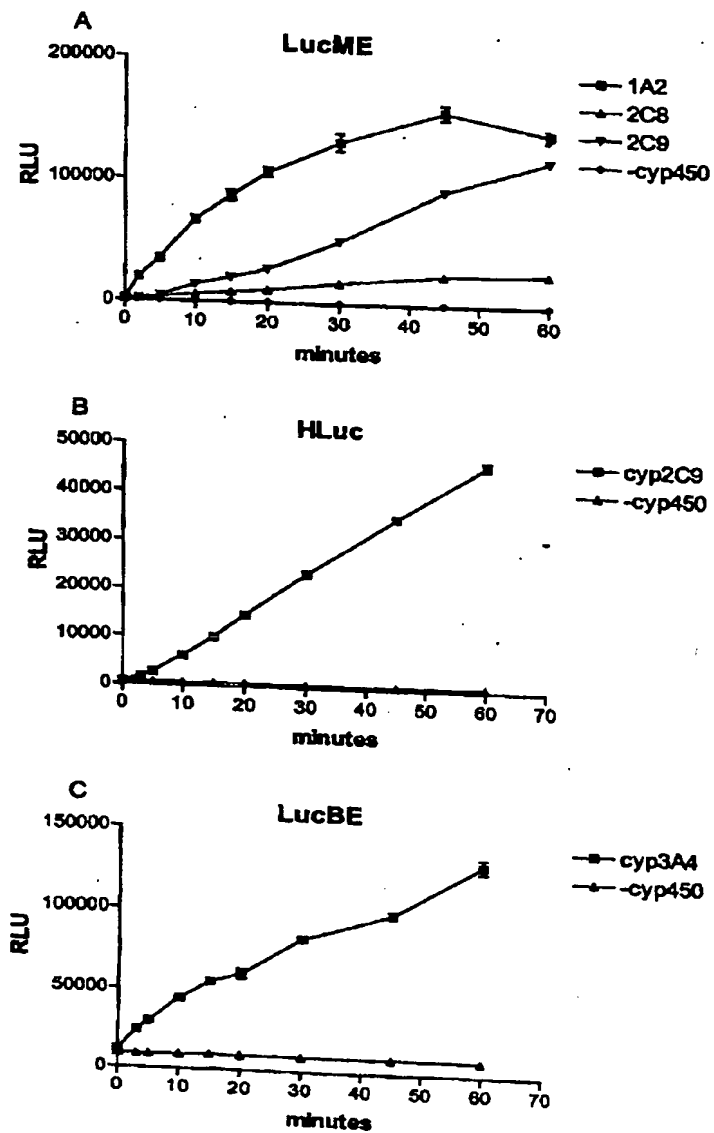
**G****H**

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**Figure 4**

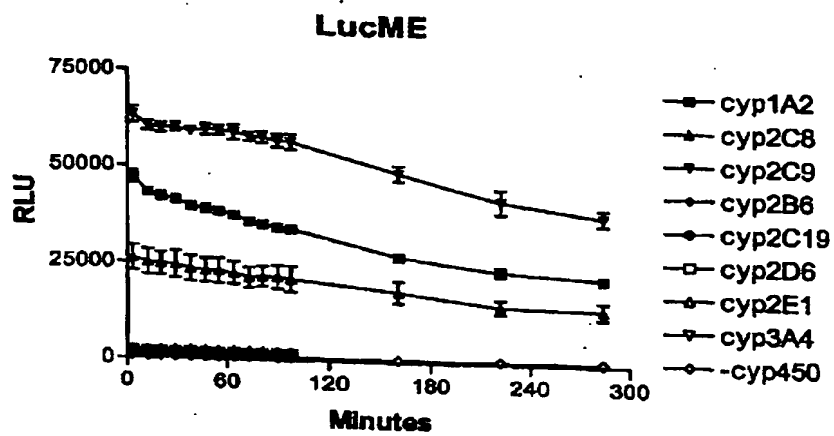
Time-dependence of cyp450/substrate incubation in two-step luminescent cyp450 reactions using D-luciferin derivatives.





**Figure 5**

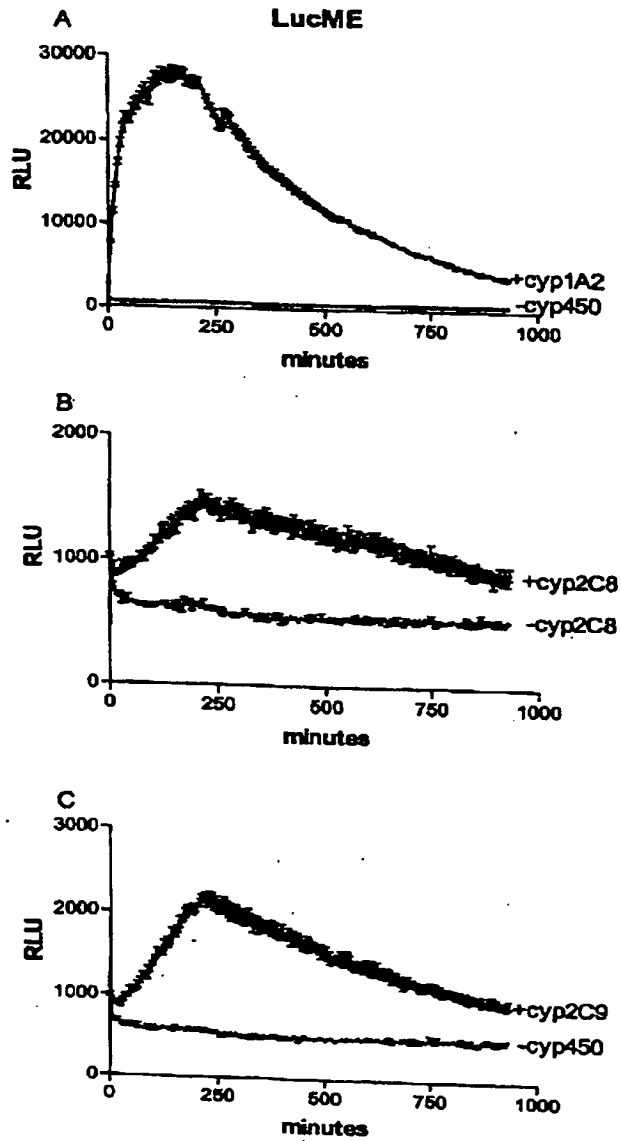
Time course of light output from two-step luminescent cyp450 reactions using LucME.



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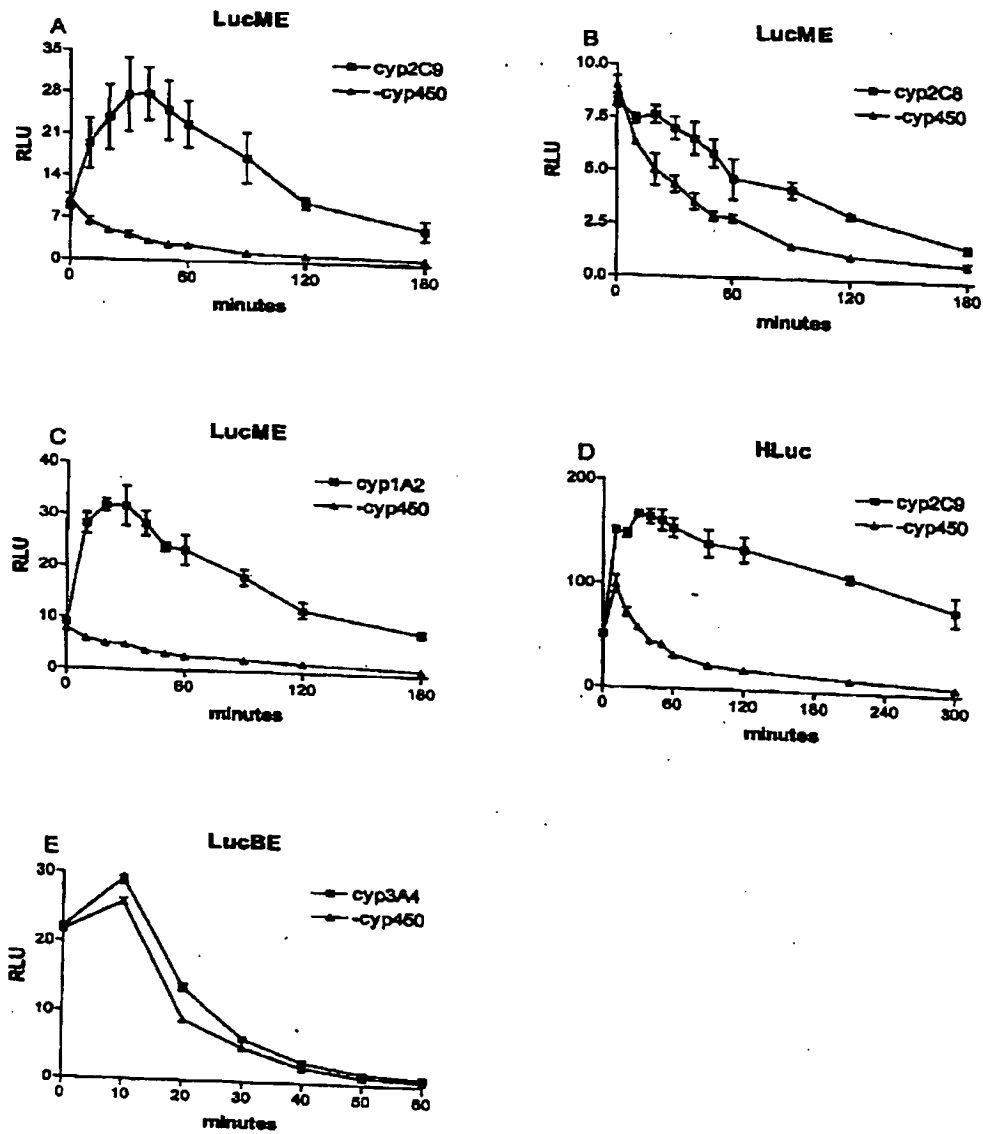
**Figure 6**

One-step luminescent cyp450 assays at room temperature using LucME.



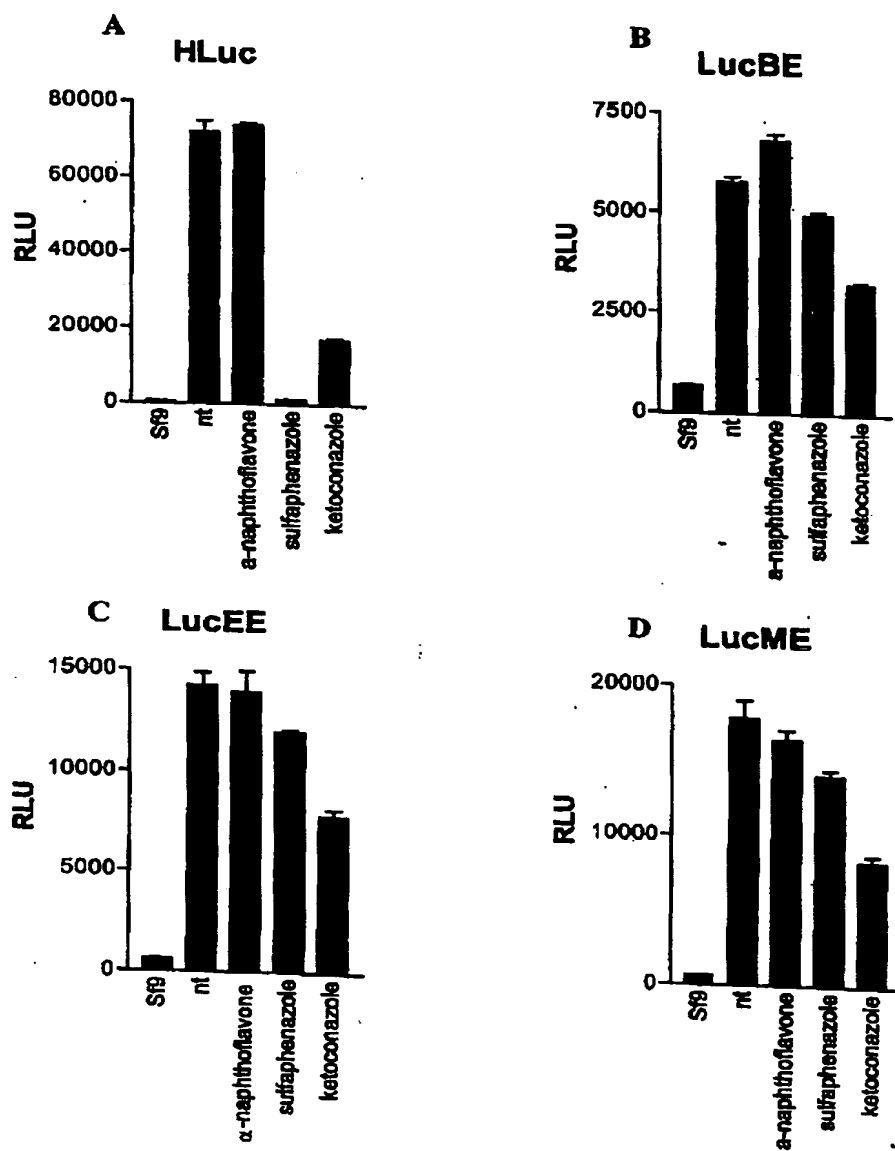
**Figure 7**

One-step luminescent cyp450 assays at 37°C using D-luciferin derivatives.



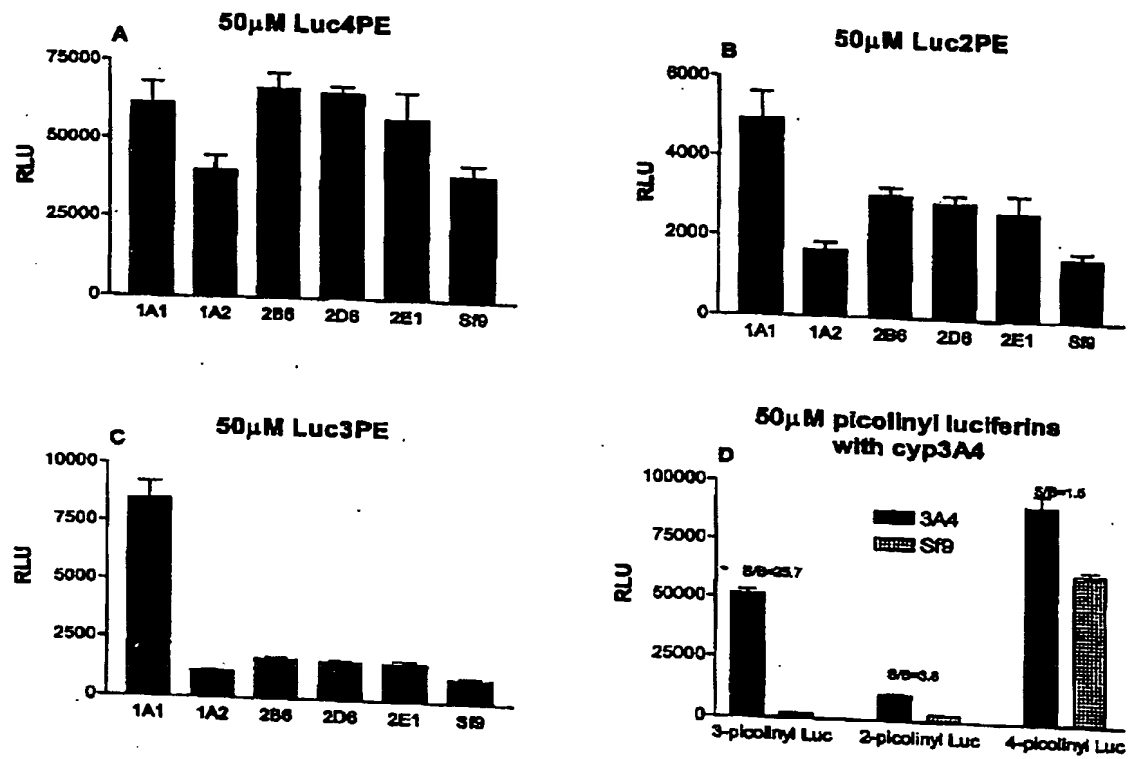
**Figure 8**

Pooled human liver microsomes in two-step luminescent cyp450 reactions using D-luciferin derivatives.



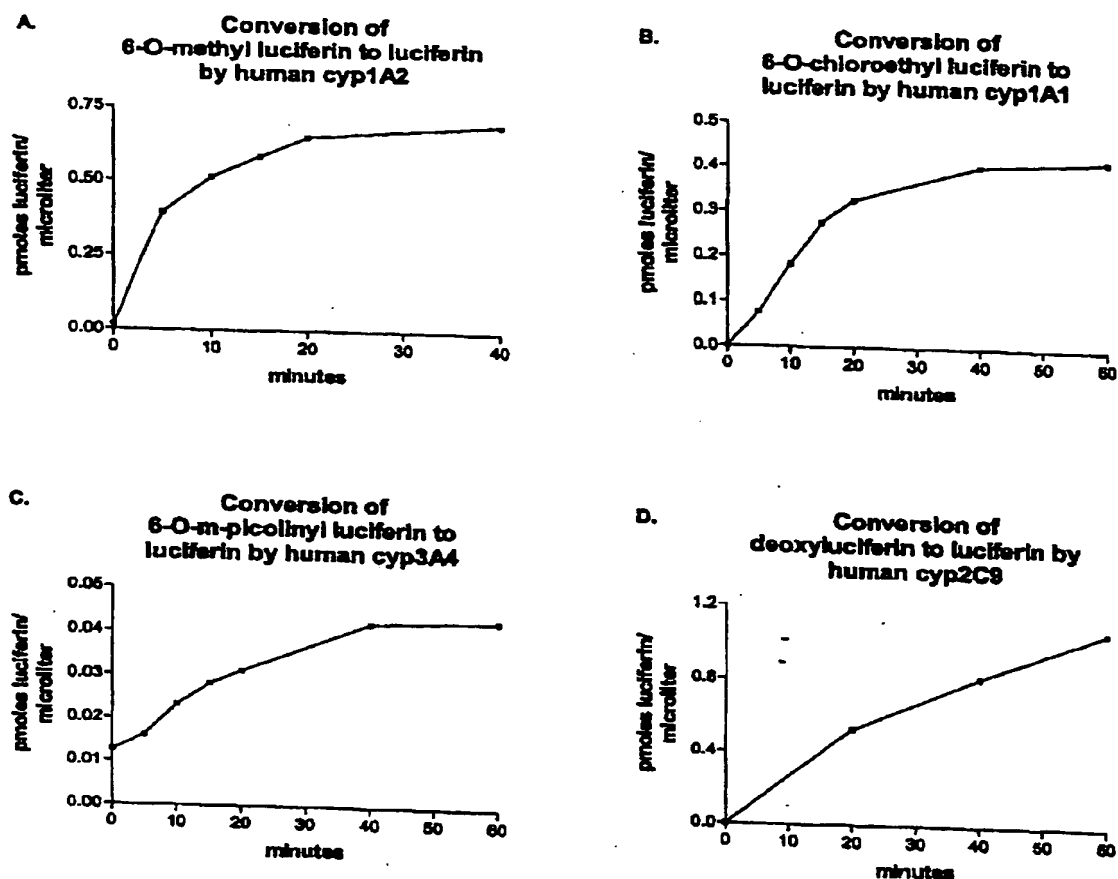
**Figure 9**

Two-step detection of de-picolinylase activity picolinyl D-luciferin derivatives



**Figure 10**

Conversion of D-luciferin derivatives to luciferin by human cyp450s

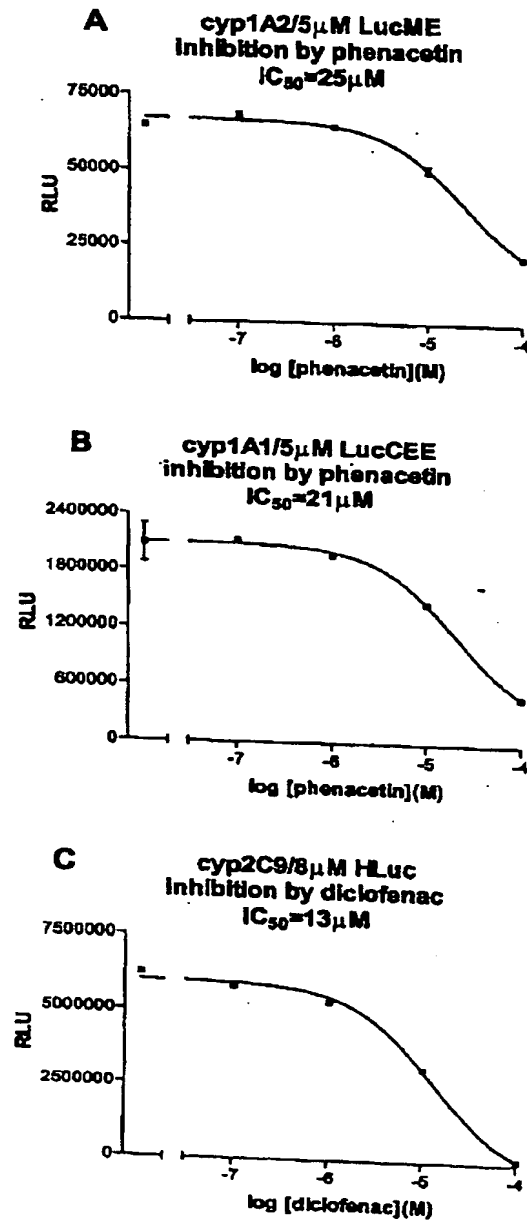


100 micromolar 6-O-methyl luciferin (panel A.), 100 micromolar 6-O-chloroethyl luciferin (panel B.), 25 micromolar 6-O-m-picolinyl luciferin (panel C.) or 100 micromolar deoxyluciferin (panel D.), was incubated with cyp1A2, cyp1A1, cyp3A4 or cyp2C9, respectively, in reaction volumes of 150 microliters at 37°C. At the indicated times reactions were stopped by addition of tert-butyl alcohol to 0.1% (v/v) and flash freezing in liquid nitrogen. 95 microliters of each reaction was subjected to fractionation by HPLC and luciferin was detected by fluorescence. The zero time points were determined by inactivating the cyp450 with 0.1% tert-butyl alcohol prior to substrate addition (cyp1A1, 1A2 and 3A4) or by determining the luciferin content of a no enzyme control with deoxyluciferin (cyp2C9).

**HPLC method:** High-pressure liquid chromatography was performed on an HP 1050 LC system equipped with a multi-wavelength absorbance (HP 1050 MWD) and fluorescence detector (HP 1046A). Separation was achieved on a 5 micron Adsorbosphere HS C18 column (Alltech Associates) with a solvent gradient of 0.05M  $\text{KH}_2\text{PO}_4$  / pH 6 (solvent A) and 80:20 acetonitrile/water (solvent B). The gradient conditions used were 15%B to 95%B over 10 min. Substrates were detected by absorbance at either 282 or 330 nm and Luciferin was detected by fluorescence at 520 nm (emission) with an excitation wavelength of 330 nm.

**Figure 11**

Inhibition of cyp450 by known cyp450 substrates.



**Figure 12**

P450 action on methoxy-coelenterazine HH, coelenterazine HH, and coelenterazine by chemiluminescent and bioluminescent detection.

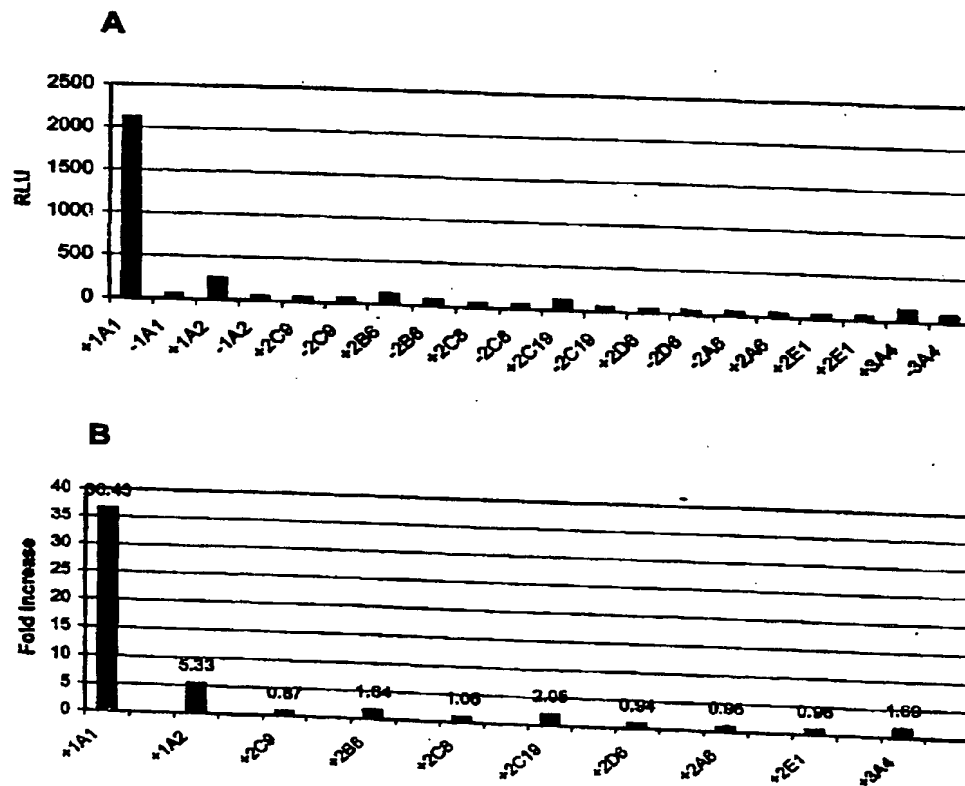




Figure 12 cont.

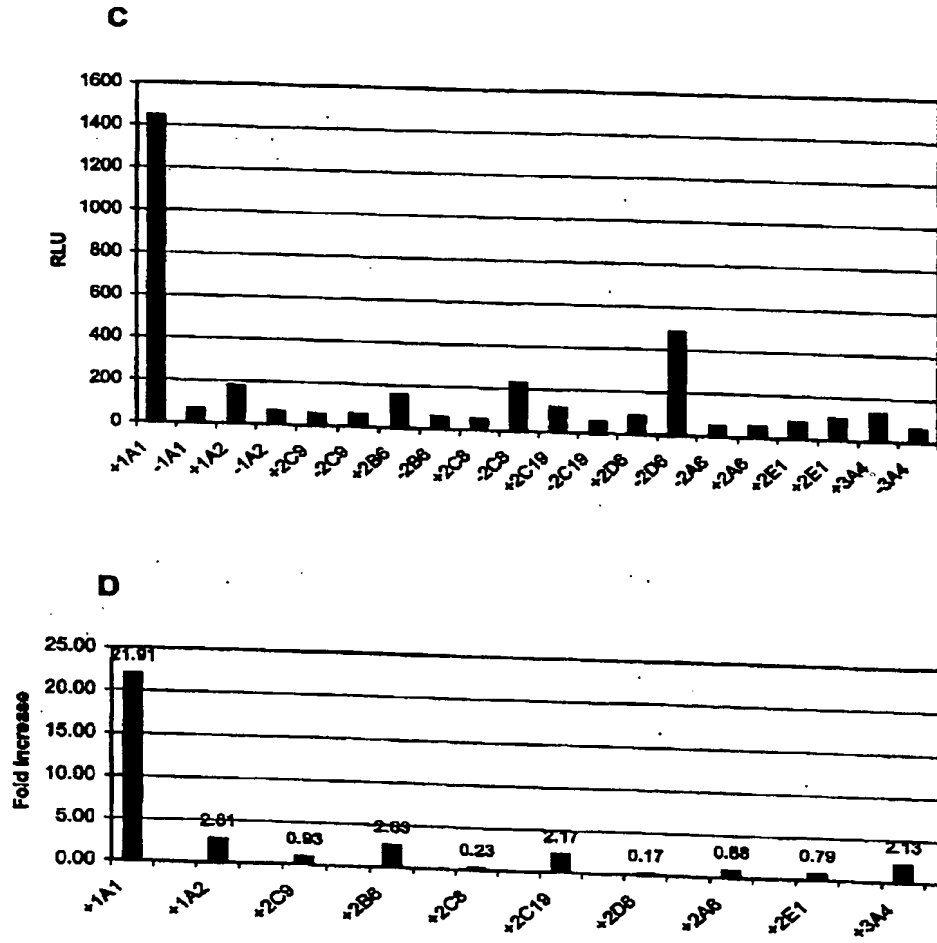


Figure 12 cont.

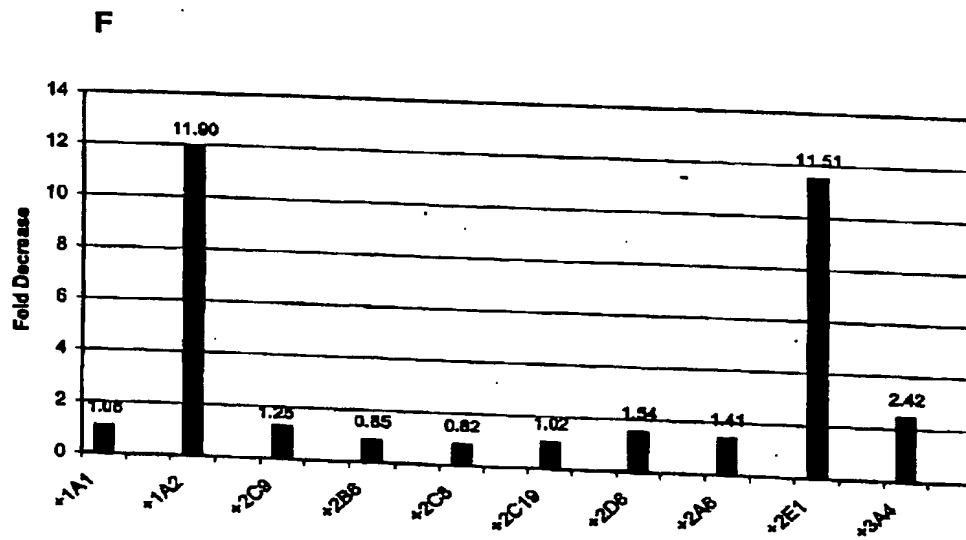
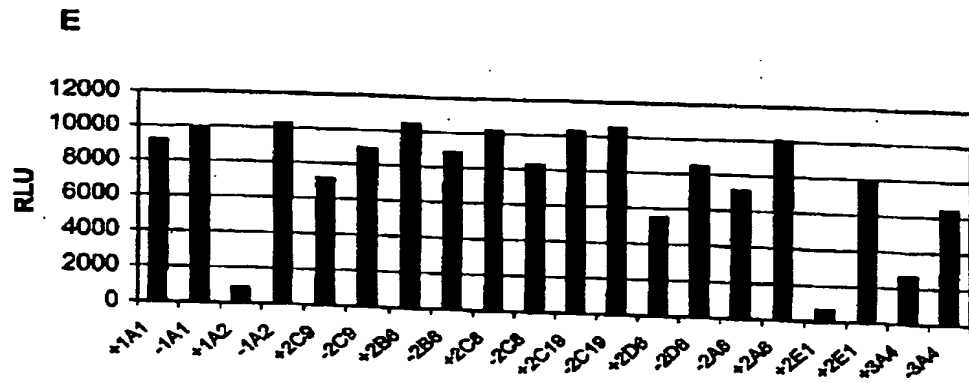


Figure 12 cont.

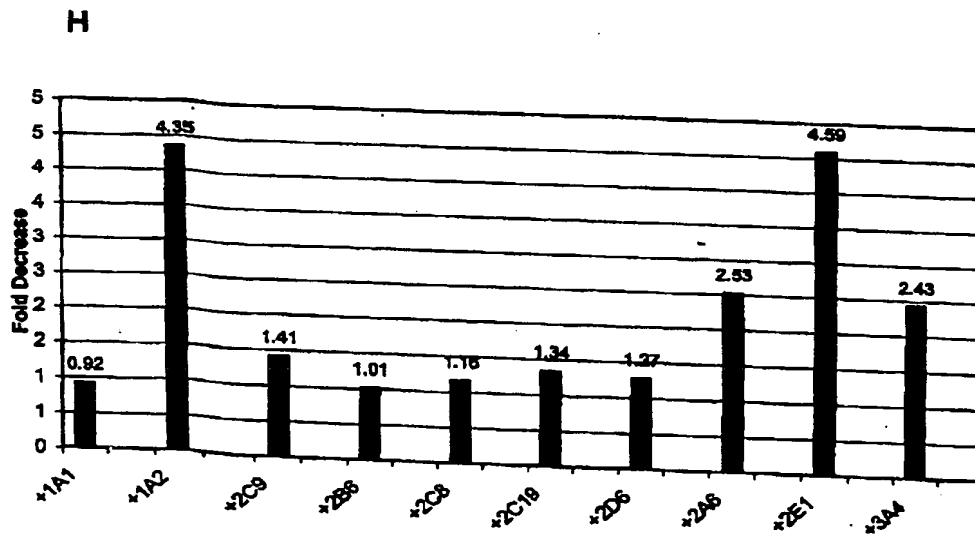
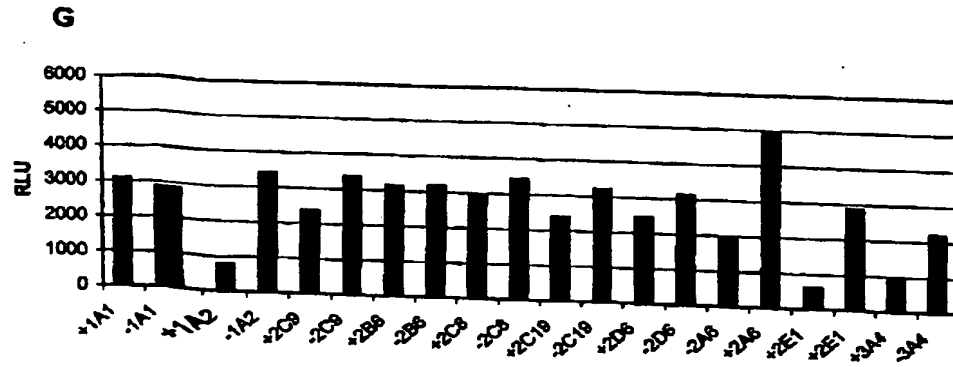


Figure 12 cont.

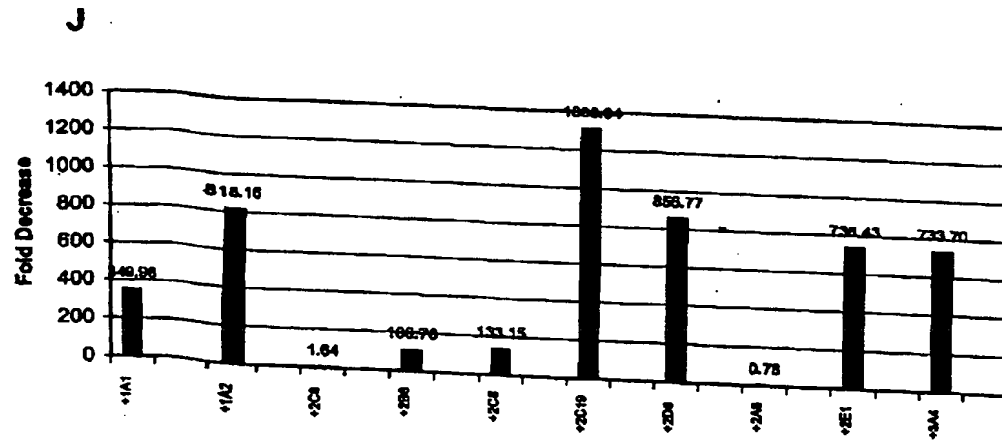
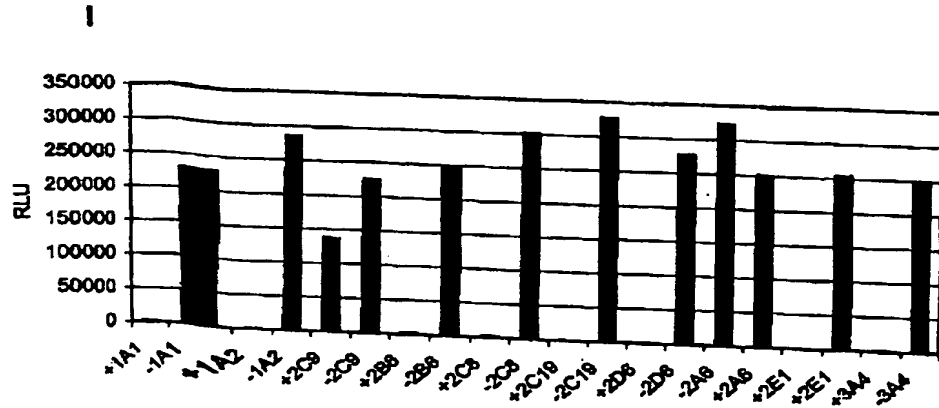


Figure 12 cont.

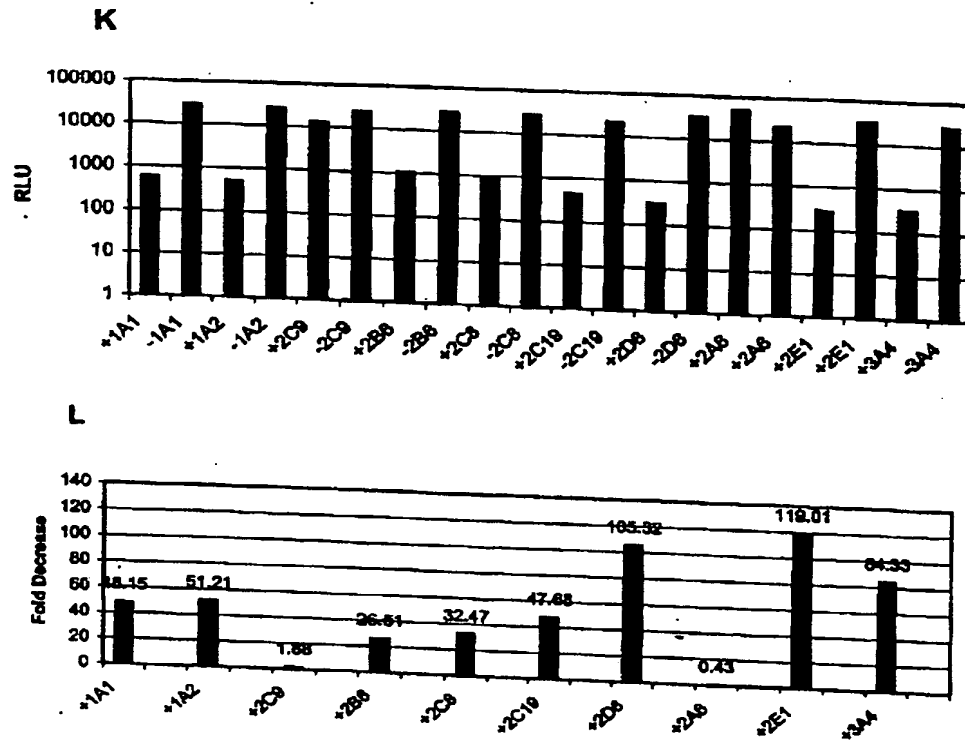


Fig. 13: Pyrophosphatase Activity

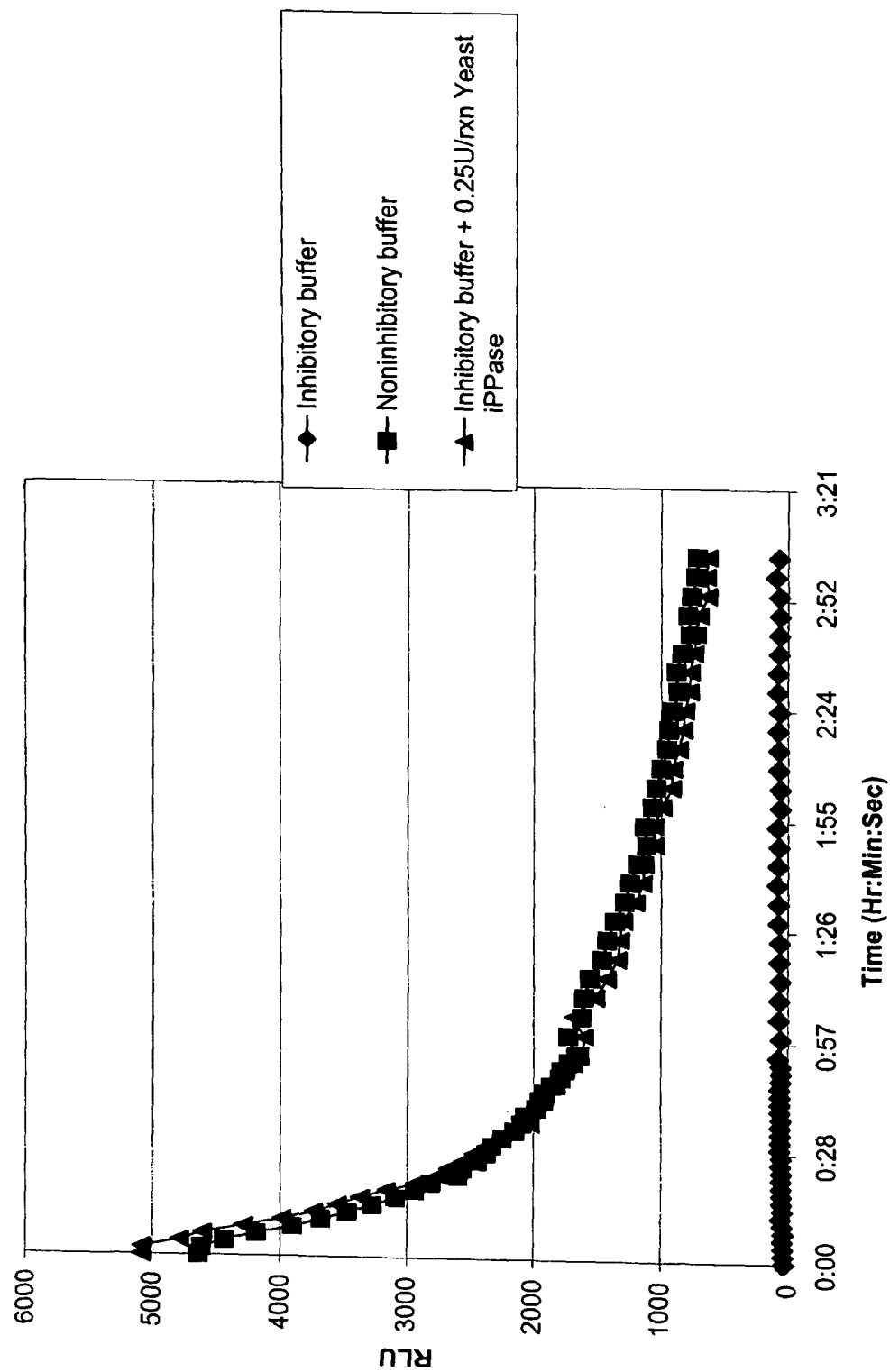


Figure 14

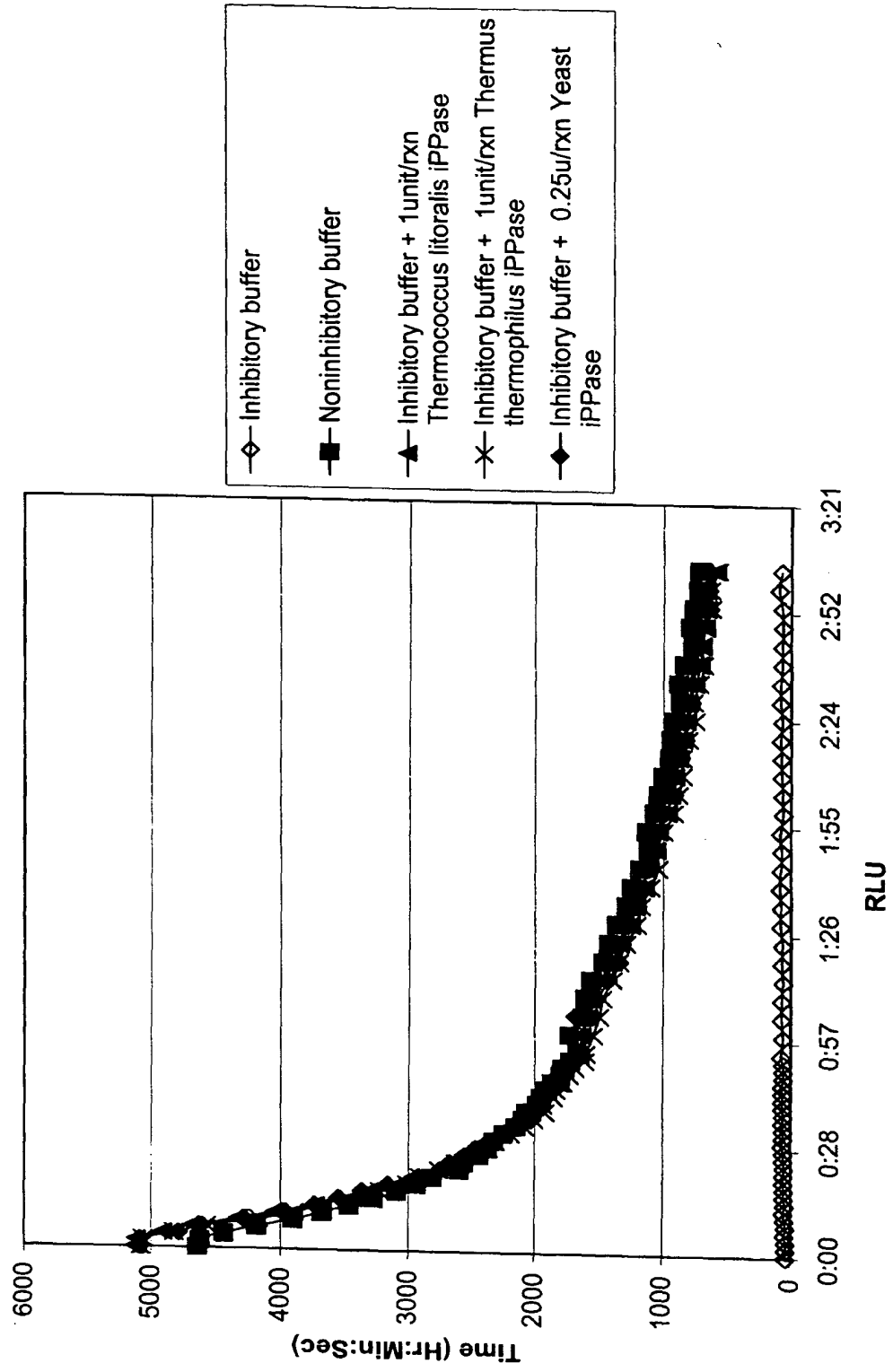


Figure 15

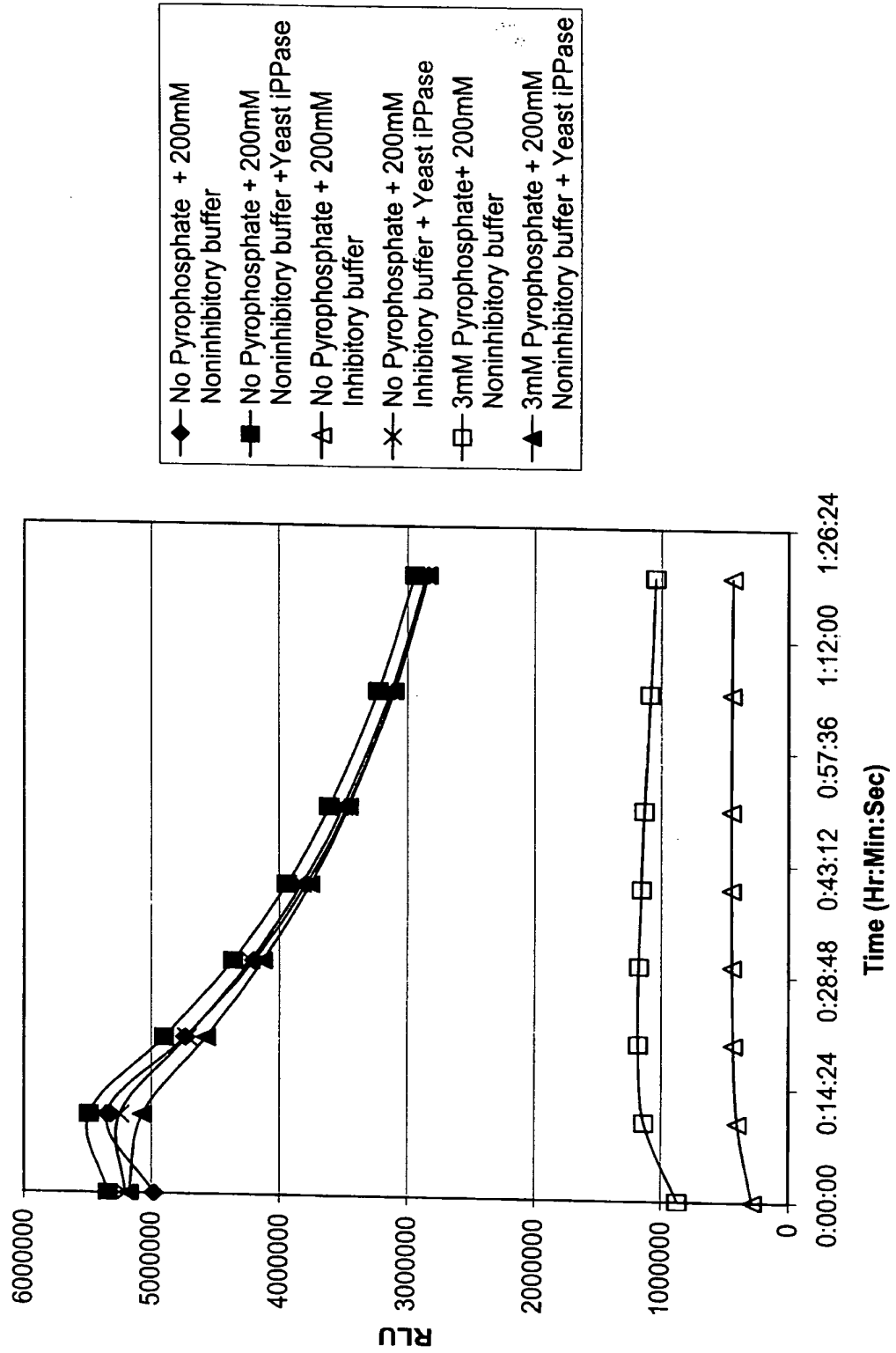




Figure 16

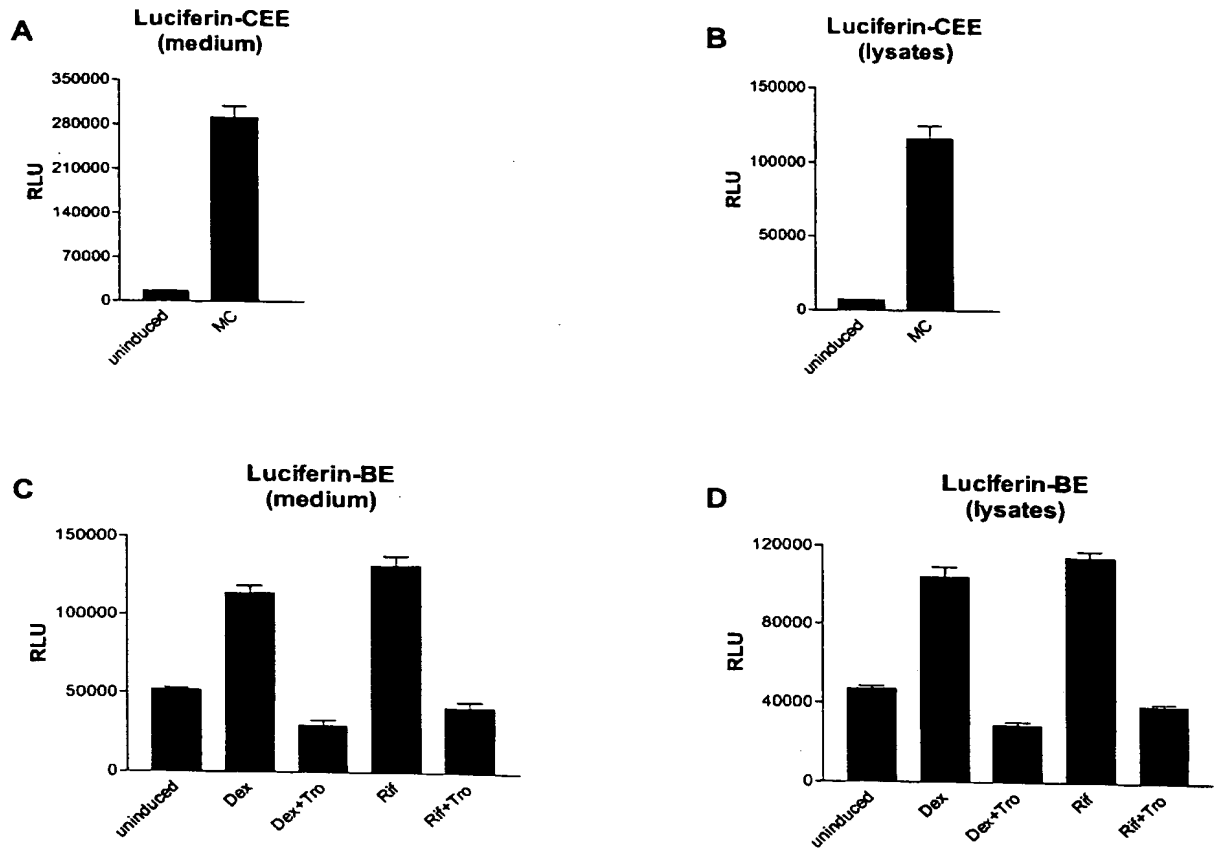


Figure 17

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**Stabilization of luminescent  
CYP1A1 signal with APMBT  
and AMBT**

